

# Mouse Dermal Microvascular Endothelial Cells

#### **Order Information**

**Product Name:** Mouse Dermal Microvascular Endothelial Cells

(mDerMENCs)

Catalogue Number: cAP-m0004

**Product Format:** Proliferating culture

Cell Number: > 90% confluent in T25 flask

#### **General Information**

mDerMENCs (cAP-m0004) are pooled cells isolated from skin tissues of C57BL6 mice. mDerMENCs are shipped in proliferating culture with >90 confluence (the cells are provided @ passage 2). ENDO-Growth medium (contains 5% serum and growth supplements, Cat#cAP-02) is recommended for cell culture and these cells have a guaranteed average additional population doubling levels 6-8 when cultured following the detailed protocol described below).

#### Characterization of the cells

Cytoplasmic VWF / Factor VIII: >95% positive by immunofluorescence
Cytoplasmic uptake of Di-I-Ac-LDL: >95% positive by immunofluorescence
Cytoplasmic PECAM1 >95% positive by immunofluorescence

mDerMENCs are negative for bacteria, yeast, fungi, and mycoplasma.

**Product Use: mDerMENCs** are for research use only.

**Shipping:** Proliferating culture in T25 flask.

## **Handling of Arriving Cells**

When you receive the cells, leave the flask in a 37°C CO2 incubator for 1 hour first, and then replace the transport medium with fresh ENDO-Growth medium. Let the cells grow for 24 hours before subculture.

### 1. Subculture Protocol:

- A) Coating T25 flasks: Add 2ml of Quick Coating Solution (**cAP-01**) into one T25 flask and make sure all surface area of the flask is covered by coating solution. Five minutes later, dispose Quick Coating Solution by aspiration and the flask is ready to be used (no need for overnight incubation when coated with Quick Coating Solution).
- B) Rinse the cells in T25 flask with 5ml PBS (Room Temperature, RT) twice.

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- C) Add 2ml of Trypsin/EDTA (**RT**) (Invitrogen: Cat No: 25300-062) into T25 flask (make sure all surface area of the T25 flask is covered with Trypsin/EDTA), and gently dispose the Trypsin/EDTA solution **within 10-15 seconds** with aspiration.
- D) Leave the T25 flask with the cells at **RT** for 1-2 minute (cells normally come off the surface within 1 minute, check under microscope).
- E) Suspend the cells with 20ml of ENDO-Growth medium and the cell suspension is transferred directly into 2 x pre-coated T25 flasks (10ml each, and the cells are subcultured at 1:2 ratio)

(Note: No need spin the cells during the subculture process).

## 2. Cell culture protocol (proliferating):

- A) Culture medium (ENDO-Growth medium) is changed every 2 days.
- B) The cells normally become confluent within 7 days (when split at a 1:4 ratio).

## 3. Preparation of quiescent cells:

A) ENDO-Basal medium (cAP-03) containing 0.5% FBS is used to induce quiescent endothelial cells (after 12-18hours).

#### Other useful information

Items	Company	Cat #
Quick Coating Solution	Angio-Proteomie	cAP-01
ENDO-Growth medium	Angio-Proteomie	cAP-02
ENDO-Basal medium	Angio-Proteomie	cAP-03
<b>ENDO-Growth Supplement</b>	Angio-Proteomie	cAP-04
PBS	Invitrogen	10010
Trypsin/EDTA	Invitrogen	25300-062

Caution: Although mouse endothelial cells are isolated from laboratory mice testing pathogen free, users should handle these cells with caution, no test can completely guarantee the absence of infectious agents. Prompt protective measures should always be taken when users are handling these cells.